

THE CONSTITUENTS
OF THE BULB OF
BUPHANE DISTICHA

BY
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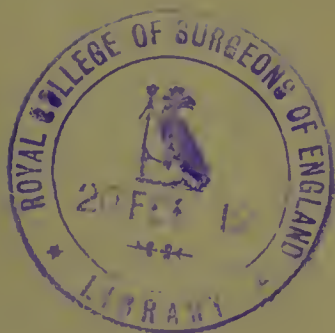


THE WELLCOME CHEMICAL RESEARCH LABORATORIES

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CXXXIII.—*The Constituents of the Bulb of
Buphane disticha.*

By FRANK TUTIN.

Buphane disticha, Herb. (formerly known as *Boophane toxicaria*, Herb.), is a bulbous plant belonging to the *Amaryllidaceae*. It is a native of South Africa, where it is known as the "poison bulb," or, to the Dutch, as "gift-bol," and is a frequent, although nowhere abundant, object on the Veldt. The plant, which is stated to have been used as an arrow poison, and in the treatment of the disease known as red-water (Smith, *A Contribution to South African Materia Medica*, 3rd Ed., pp. 158 and 180), has long been known to possess toxic properties, but it does not appear to have been chemically examined. It has, however, been stated to contain brucine, but this has since been denied, it being affirmed that the alkaloid was aconitine (Oliver, *Chemist and Druggist*, 1908, 72, 140).

The present investigation has shown that neither brucine nor aconitine is present in *Buphane disticha*, but that the principal alkaloid is an amorphous base, designated as *buphanine*, which resembles hyoscyne in its physiological action. At least three other alkaloids are present, one of which has been identified as narcissine

(Ewins, Trans., 1910, **97**, 2406). The unusually large amount of copper which is present in the bulb may possibly also contribute to its poisonous properties.

EXPERIMENTAL.

The material employed in this investigation consisted of the bulbs of *Buphane disticha*, Herb., which varied in weight from 140 to 500 grams, and had been specially collected in South Africa for the purpose.

The dried inner portion of the bulb was found to contain a considerable amount of an alkaloid, whereas the dead, outer layers were practically free from such a substance.* On this account, only the inner portions of the bulbs were employed for this investigation.

A quantity (3.97 kilograms) of the dried inner portions of the bulbs, representing about 54.5 kilograms of the entire fresh bulbs, were completely extracted by continuous percolation with hot alcohol. After removing the greater part of the solvent, a dark-coloured extract was obtained, which amounted to 766 grams. The whole of this was mixed with water, and steam passed through the mixture for several hours. The distillate, which contained some drops of oil, was extracted with ether. This removed about 2 grams of an essential oil containing furfuraldehyde, together with a small amount of an acid having the odour of valeric acid.

After the distillation of the extract with steam, as above described, there remained a dark-coloured aqueous liquid (A), together with a quantity of a black resin (B). These products were separated while still hot, the resin being repeatedly washed with hot water.

Examination of the Aqueous Liquid (A).

The aqueous liquid (A), on cooling, deposited a relatively small amount of a brown, amorphous product. Nothing definite could be isolated from this solid, and, on ignition, it left an appreciable proportion of residue, consisting chiefly of copper oxide.

Isolation of Acetovanillone.

The filtered aqueous liquid was then extracted six times with ether, the ethereal liquids being washed and concentrated. The ethereal solution so obtained, which contained no alkaloid, was

* In this connexion it may be noted that the dry, outer layers of the bulb, which are of a fine, silky texture, are used by the natives of South Africa as a substitute for surgical bandages.

extracted successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide. The ammonium carbonate extract yielded nothing, but on acidifying the sodium carbonate extracts and again extracting with ether, about 1.5 grams of a product were obtained, which, when distilled under diminished pressure and crystallised from ethyl acetate, proved to be acetovanillone (m. p. 115°. Found, C=65.0; H=6.1. Calc., C=65.1; H=6.0 per cent.).

The occurrence of this substance in Nature appears heretofore only to have been observed in the rhizome of *Apocynum cannabinum*, Linné, and *A. androsaemifolium*, Linné (compare Trans., 1908, **93**, 1513; 1909, **95**, 2863).

The potassium hydroxide extract of the ethereal liquid, from which the acetovanillone had been removed, yielded only a little resinous matter, but, on finally evaporating the ethereal solution, a small amount of a crystalline, neutral substance was obtained. This compound was sparingly soluble in ethyl acetate, from which it separated in colourless needles, melting at 245—250°.

The aqueous liquid which had been extracted with ether was then shaken repeatedly with amyl alcohol. The liquid so obtained contained a considerable amount of alkaloidal material, which was removed by repeated extraction with dilute hydrochloric acid. The amyl-alcoholic solution yielded only a very small amount of resinous matter.

Separation of the Alkaloids.

The original aqueous liquid which had been extracted with amyl alcohol was rendered alkaline by the addition of sodium carbonate, and extracted many times with ether, but, as this treatment failed to remove the whole of the alkaloidal material, the alkaline liquid was subsequently extracted with amyl alcohol. The bases contained in the acid, aqueous liquid obtained from the first amyl-alcoholic solution, as above described, were then similarly separated into two portions, one dissolved by ether and the other by amyl alcohol.

The entire amount of bases soluble in ether, dissolved in this solvent, was then extracted with successive portions (20 c.c. each) of 1 per cent. hydrochloric acid. The first eleven extracts were brownish-coloured, slightly alkaline liquids, but the twelfth extraction yielded an acid liquid. The strength of the hydrochloric acid employed for the extraction was then increased to 5 per cent., when, after five more extractions, all the alkaloid had been removed from the ethereal liquid. All the liquids obtained by extraction with 1 per cent. hydrochloric acid yielded, on slow evaporation, only viscous syrups, but the five extracts obtained by

means of the stronger acid all deposited, on keeping, a quantity of solid in the form of wart-like granules. This solid was the hydrochloride of an alkaloid, but it could not be obtained in a definite, crystalline form, nor could any crystalline derivative be prepared from it.

It is thus evident that the alkaloids soluble in ether consisted of at least two bases, one of which was much more strongly basic than the other, and formed by far the greater part of the mixture. This strongly basic product could not be crystallised, nor could any of its salts, but it evidently consisted essentially of a single compound, since, as shown below, it is readily changed by potassium hydroxide, giving a very good yield of a crystalline base. In view of this fact it is considered appropriate to designate it *buphanine*, although no evidence regarding its formula was obtained. Its crystalline hydrolytic product may then be named *buphanitine*.

Formation of Buphanitine, $C_{23}H_{24}O_6N_2$.

A quantity of the amorphous buphanine was heated for two hours on a water-bath with a large excess of alcoholic potassium hydroxide. The greater part of the solvent was then removed, the mixture poured into water, and the base extracted with chloroform. The residue obtained on removing the chloroform was dissolved in alcohol, when it quickly crystallised in colourless prisms, which, on heating at 130° , lost solvent of crystallisation, and then melted at 240° :

0.5573,* on heating at 130° , lost 0.0599 EtOH. EtOH=10.7.

0.0931 † gave 0.2210 CO_2 and 0.0474 H_2O . C=64.7; H=5.6.

0.3915 † „ 22.1 c.c. N_2 (moist) at 22° and 780 mm. N=6.6.

$C_{23}H_{24}O_6N_2$.EtOH requires EtOH=10.7 per cent.

$C_{23}H_{24}O_6N_2$ requires C=65.0; H=5.6; N=6.6 per cent.

Buphanitine is very readily soluble in chloroform or ethyl acetate, moderately so in hot alcohol or boiling water, but sparingly soluble in the latter solvents when cold. It cannot be crystallised from water, but separates from this solvent in the form of a jelly.

Buphanitine Hydrochloride, $C_{23}H_{24}O_6N_2.HCl$.—This salt, prepared by adding hydrochloric acid to an alcoholic solution of the base, crystallised readily from a mixture of ethyl acetate and alcohol in small, colourless needles. It melted and decomposed at $265-268^\circ$:

0.1815 gave 0.0581 AgCl. Cl=7.9.

$C_{23}H_{24}O_6N_2.HCl$ requires Cl=7.7 per cent.

* Air-dried substance.

† Dried at 130° .

Buphanitine Methiodide, $C_{23}H_{24}O_6N_2, CH_3I$.—This derivative was quickly formed on adding methyl iodide to an alcoholic solution of the base. It crystallised readily from slightly diluted alcohol in slender, glistening prisms, which melted and decomposed at 278° :

0.1582 gave 0.2944 CO_2 and 0.0740 H_2O . $C=50.7$; $H=5.2$.

$C_{23}H_{24}O_6N_2, CH_3I$ requires $C=50.9$; $H=4.8$ per cent.

The alkaline aqueous liquid from which the buphanitine had been removed by means of chloroform was found to contain no acid, but only a very small amount of brown resin. It is thus evident that buphanine is not an ester of buphanitine, and it would appear probable that the latter is formed by the addition of one or more molecules of water to the former.

Isolation of Narcissine, $C_{16}H_{17}O_4N$.

The amyl-alcoholic solution of the bases which were not removed by extraction with ether, as previously described, was evaporated to a low bulk, freed from a small amount of amorphous, non-basic material, and the filtered liquid poured into a large volume of ether. The clear mixture was then washed with water, when a relatively small amount of an alkaloid was removed. This base could not be crystallised, nor could any crystalline salt be obtained from it. The ether-amyl alcoholic liquid was then kept for some time, when a small amount of narcissine separated (m. p. 267°). Found, $C=67.1$; $H=6.0$. Calc., $C=66.9$; $H=5.9$ per cent.).

A direct comparison of this base with a specimen of narcissine obtained from the bulb of the common daffodil, kindly supplied by Mr. A. J. Ewins (compare Trans., 1910, **97**, 2406), confirmed the identity of the two.

Narcissine picrate, $C_{16}H_{17}O_4N, C_6H_3O_7N_3$, has not previously been described. It crystallises readily in bright yellow plates, which decompose and melt at 196 — 199° .

The liquid from which the narcissine had separated was fractionally extracted with hydrochloric acid in a manner similar to that already described, when it was resolved into two products of varying affinity for acid, but the weakly basic material was very small in amount. The first four fractions obtained by shaking with dilute hydrochloric acid were alkaline, and, on keeping, deposited some narcissine, which was removed. The filtrates from this base then yielded small amounts of narcissine hydrochloride, but the greater part of the material contained in them was amorphous, and consisted chiefly of buphanine, since it gave buphanitine on hydrolysis.

Isolation of Chelidonic Acid, C₇H₄O₆.

The alkaline aqueous liquid which had been deprived of alkaloids by means of ether and amyl alcohol, as previously described, was rendered nearly neutral by the addition of hydrochloric acid, and acidified with acetic acid; it was then evaporated under diminished pressure to a small bulk. During this operation about 20 grams of cuprous oxide were deposited. It is evident from this that an unusually large amount of copper was present in the bulb under examination. After removal of the cuprous oxide, the liquid was further evaporated to a syrup, when it was mixed with much hot alcohol, and kept for a few hours. The dark greenish-brown solid which had separated was collected, and washed with dilute alcohol, when it was found to contain an appreciable amount of organic matter, together with copper, sodium, and chlorine. It was dissolved in hot, very dilute alcohol, from which it separated, on cooling, as a brown, partly crystalline powder, free from halogen, although it still contained copper and sodium. This partly purified product was then submitted to a very prolonged extraction in a Soxhlet apparatus with 80 per cent. alcohol, when a colourless solid was slowly removed. The latter was crystallised four times from dilute aqueous hydrochloric acid, when large, colourless, glistening needles of the acid sodium salt of chelidonic acid were obtained (compare Willstätter and Pummerer, *Ber.*, 1904, **37**, 3744). The salt contained water of crystallisation:

0.0942 gave 0.1282 CO₂, 0.0249 H₂O, and 0.0207 Na₂CO₃. C=38.6;
H=2.9; Na=2.2.

C₇H₃O₆Na.C₇H₄O₆.2H₂O requires C=38.6; H=2.8;
Na=2.4 per cent.

The above-mentioned compound was converted, through the silver salt, into chelidonic acid, which melted and decomposed at 262°. (Found, C=45.5; H=2.3. Calc., C=45.6; H=2.2 per cent.)

The original aqueous-alcoholic filtrate from the crude acid sodium salt of chelidonic acid was fully examined, but nothing crystalline could be separated from it. It readily yielded *d*-phenylglucosazone, and therefore evidently contained sugar. A quantity of it was evaporated as far as possible, and acetylated by means of acetic anhydride, after which the acetyl derivative was dissolved in ether, and freed from a considerable proportion of acidic, resinous matter by means of aqueous alkalis. The purified acetyl derivative, which did not crystallise, was then hydrolysed by means of dilute aqueous sulphuric acid, when a sugar was obtained which could not be crystallised, but, since it was strongly laevorotatory and yielded *d*-phenylglucosazone, it evidently consisted essentially of levulose.

Examination of the Resin (B).

The resin (B) was a black, wax-like solid, and amounted to 85 grams. It was dissolved in alcohol, and mixed with purified sawdust, after which it was extracted successively in a large Soxhlet apparatus with petroleum (b. p. 35—60°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

The petroleum extract was a black, wax-like mass, and amounted to 56 grams. It was dissolved in ether, and shaken with aqueous ammonium carbonate, when an emulsion was formed, but nothing was dissolved by the alkali. The ethereal solution was separated from the emulsion, and the latter washed repeatedly with ether, after which air was passed through it for some time, when a brown powder separated. This was collected, but it could not be purified by crystallisation, and evidently contained much resinous matter. It appeared, however, also to contain some hydrocarbon, and it was therefore dissolved in hot glacial acetic acid, and deprived of resin by oxidation with a hot solution of potassium permanganate in slightly diluted acetic acid. The mixture was then treated with sulphur dioxide, when it yielded a substance which, after distillation under diminished pressure and crystallisation from ethyl acetate, melted at 75°, and was recognised as pentatriacontane. (Found, C=85.1; H=14.5. Calc., C=85.4; H=14.6 per cent.)

The ethereal liquid from which the emulsion had been separated was then shaken with aqueous potassium carbonate, which removed some fatty acids, and also gave rise to the formation of an emulsion. The fatty acids were converted into their ethyl esters, the latter being distilled under diminished pressure. These esters of the free fatty acids were examined, together with a similar product subsequently obtained from the combined acids.

Isolation of a Phytosterol, C₂₇H₄₆O.

The ethereal liquid which had been deprived of free fatty acids, as above described, was shaken with a solution of potassium hydroxide, but nothing was removed by this treatment. The ether was then evaporated, and the residue heated for two hours with an excess of alcoholic potash, after which the liquid was poured into water, and extracted many times with ether. The ethereal liquid so obtained yielded, on evaporation, a further quantity of pentatriacontane. The mother liquors from this hydrocarbon then gave a product which, on fractional crystallisation from various solvents,

formed long, flattened needles, melting at 132° . This substance, which amounted to about 0.4 gram, gave colour reactions which indicated it to be a phytosterol:

0.1351,* on heating at 110° , lost 0.0068 H_2O . $\text{H}_2\text{O}=5.0$.

0.1233 † gave 0.3804 CO_2 and 0.1306 H_2O . $\text{C}=84.1$; $\text{H}=11.7$.

$\text{C}_{27}\text{H}_{46}\text{O}$, H_2O requires $\text{H}_2\text{O}=4.5$ per cent.

$\text{C}_{27}\text{H}_{46}\text{O}$ requires $\text{C}=84.3$; $\text{H}=11.5$ per cent.

The final mother liquors from which the phytosterol had been obtained yielded only some yellow, uncrystallisable oil of high boiling point.

The alkaline aqueous liquid, from which the phytosterol and hydrocarbon had been removed, was acidified, and extracted with ether, when a quantity of fatty acids was obtained. These were converted into their ethyl esters, and the latter distilled under diminished pressure. The acids obtained from these esters yielded, on crystallisation from ethyl acetate, a mixture of palmitic and stearic acids (m. p. 58°), whilst the mother liquors from this mixture contained some unsaturated acid. A similar result was obtained on examining the acids yielded by the esters of the free fatty acids, previously referred to. The amount of unsaturated acids was not sufficient for their complete examination.

Examination of the Ether, Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The ethereal extract of the resin amounted to 8 grams. On concentrating its ethereal solution, a quantity of a sparingly soluble, brown solid separated. This was collected, and submitted to prolonged extraction in a Soxhlet apparatus with ethyl acetate, when a light-coloured product was obtained. The latter, after crystallisation from dilute pyridine, formed microscopic, colourless leaflets, which melted and decomposed at 286° , and was identified as ipuranol, $\text{C}_{23}\text{H}_{35}\text{O}_2(\text{OH})_2$.

The original ethereal mother liquor, from which the crude ipuranol had been separated, was exhaustively examined, but only amorphous products and a trace of pentatriacontane were obtained from it.

The chloroform, ethyl acetate, and alcoholic extracts of the resin amounted to 9, 1.6, and 5.8 grams respectively, and were found to consist entirely of dark-coloured, resinous products.

Physiological Tests.

The physiological action of the amorphous alkaloids obtained from *Buphane disticha* and that of buphanitine has been investi-

* Air-dried substance.

† Dried at 110° .

gated in the Wellcome Physiological Research Laboratories by Dr. P. P. Laidlaw, to whom the author now expresses his thanks. Narcissine had previously been investigated in this direction (Ewins, *loc. cit.*), and found to be not very toxic.

The strongly basic, amorphous alkaloid, buphanine, produces physiological effects similar to those of hyoscyne and hyoscyamine, particularly the former, but its action is weaker and less lasting than that of either of the last-mentioned bases. It is a mydriatic, and paralyses the salivary secretion and the vagus endings in the heart, and causes death by respiratory failure of central origin. Its action is antagonistic to that of pilocarpine.

Buphanitine is almost inactive, whilst the weakly basic product is a convulsant poison. The amorphous base, which is soluble in water, produced effects reminiscent of those of colchicine and narcissine.

Summary.

The results of the foregoing investigation may be summarised as follows:

The living inner portions of the bulb of *Buphane disticha*, Herb., contain an abundant amount of alkaloid, whilst the dry outer layers are free from such a substance.

An alcoholic extract of the inner portion of the bulbs, when distilled with steam, yielded a small amount of an essential oil containing furfuraldehyde.

The portion of the non-volatile products which was soluble in water yielded a small amount of acetovanillone, a quantity of chelidonic acid, and considerable amounts of copper and lævulose. It also gave a mixture of alkaloids, the principal constituent of which is an amorphous, strongly basic product, designated *buphanine*, which possesses a physiological action similar to that of hyoscyne. A weakly basic and a water-soluble alkaloid were also obtained, together with a small amount of narcissine, $C_{16}H_{17}O_4N$. The weakly basic alkaloid is a convulsant poison, whilst the base soluble in water resembles colchicine and narcissine in its physiological action.

Buphanine, on hydrolysis, is converted into a crystalline alkaloid, *buphanitine*, $C_{23}H_{24}O_6N_2$, which melts at 240° .

The portion of the original extract which was insoluble in water consisted of amorphous products, together with pentatriacontane, a phytosterol, ipuranol, $C_{23}H_{38}O_2(OH)_2$, and a mixture of fatty acids, both free and combined.

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